Defence Research and Development Canada
Recherche et développement pour la défense Canada

DEFENCE R&D DÉFENSE

The Toxicity of Mustard and Mustard Lewisite to Terrestrial Organisms

T. Miller, S. Goudey and R. Zapf-Gilje
Golder Associates Ltd

Contract Scientific Authority: J.M. McAndless (deceased)
DRDC Suffield

Contract Report
DRDC Suffield CR 2005-198
September 1998

Canada

The Toxicity of Mustard and Mustard Lewisite to Terrestrial Organisms

T. Miller, S. Goudey, and R. Zapf-Gilje
Golder Associates Ltd

Golder Assocaites Ltd
500-4260 Still Creek Drive
Burnaby, BC
V5C 6C6

Contract Number: W7702-5-0382

Contract Scientific Authority: J.M. McAndless (deceased)

Defence R&D Canada – Suffield

Contract Report

DRDC Suffield CR 2005-198

September 1998

Golder Associates Ltd.

500 - 4260 Still Creek Drive
Burnaby, British Columbia, Canada V5C 6C6
Telephone (604) 298-6623
Fax (604) 298-5253

Golder Associates

September 24, 1998 972-1948

HAZMAT Disposal
Defence Research Establishment Suffield
Box 4000
Medicine Hat, AB
T1A 8K6

Attention: John M. McAndless, Ph.D., Project Manager

RE: TOXICITY OF MUSTARD AND MUSTARD -LEWSITE TO TERRESTRIAL ORGANISMS

Dear Dr. McAndless:

Enclosed is the report, "The Toxicity of Mustard and Mustard-Lewisite to Terrestrial Organisms" commissioned by DRES. The report was a joint effort by HydroQual Laboratories Ltd. and Golder Associates Ltd.

The report details a battery of toxicity tests that were used to determine the toxic threshold of mustard and a mustard-lewisite mixture to soil-dependent organisms, including microorganisms, invertebrates and plants. The test battery included thirteen soil health index tests conducted using both water and methanol extracts and artificial and field soil.

The results for mustard and the mustard-lewisite mixture were markedly different with toxicity threshold concentrations based on nominal concentrations of 160 mg/kg for mustard and 0.067 mg/kg for the mustard-lewisite mixture. The results for mustard are consistent with what was found for the mustard-spiked sample in the Suffield Ecological Risk Assessment conducted by Golder for DRES. However, the results for the mustard-lewisite spiked soil from these laboratory experiments indicate a much lower threshold than what was observed for lewisite-contaminated soils in the field for the Suffield Ecological Risk Assessment. These results suggest that lewisite contamination in soil changes over time, resulting in significantly reduced toxicity to soil-dependent organisms.

We believe the results contained in this report represent a significant step forward in determining the threshold of these contaminants to soil-dependent receptors. However, the results are of limited usefulness with the absence of quantitative analytical chemistry to determine the exact concentrations of mustard and mustard-lewisite in the test media. Analytical chemistry is also required in order to compare the results from the standard tests presented in this report to results from other tests or test species reported in the literature.

The results from this report have generated a number of research questions that could be answered with further study. These have been identified in Section 9.0 General Recommendations.

We hope this report meets with your approval. As always, Golder Associates and HydroQual enjoyed working with DRES scientists on this interesting project and we hope to continue to work together on exciting opportunities in the future.

Yours very truly,

GOLDER ASSOCIATES LTD.

Trish Miller, M.Sc.
Senior Toxicologist

Reidar Zapf-Gilje, Ph.D., P.Eng.
Principal

HydroQual Laboratories Ltd.

Stephen Gouldey, Ph.D.
President

TAM/kb
972-1948
G:\USER\KBURNER\Rpt_let.doc

Golder Associates Ltd.

500 - 4260 Still Creek Drive
Burnaby, British Columbia, Canada V5C 6C6
Telephone (604) 298-6623
Fax (604) 298-5253

Golder Associates

REPORT ON

THE TOXICITY OF MUSTARD AND MUSTARD LEWISITE TO TERRESTRIAL ORGANISMS

Submitted to:

HAZMAT Disposal
Defence Research Establishment Suffield
Box 4000
Medicine Hat, AB
T1A 8K6

DISTRIBUTION:

2 Copies - Defence Research Establishment Suffield
2 Copies - Golder Associates Ltd.
2 Copies - HydroQual Laboratories Ltd.

September 1998 972-1948

EXECUTIVE SUMMARY

Chemical warfare agents, specifically mustard (HD) and mustard-lewisite (HL) mixtures have been used and stored in defence research and training establishments in Canada and abroad. HydroQual Laboratories Ltd. was contracted through Golder Associates Ltd. (Burnaby) to evaluate the toxicity of HD and HL to soil-dwelling organisms for Defence Research Establishment Suffield (DRES). The toxicity of HD and HL to terrestrial organisms was evaluated by applying soil health index tests (SHI) to two types of soils fortified with known quantities of HD and HL. Several concentrations were used to establish a dose-response relationship. The tests included root elongation and seedling emergence (lettuce, alfalfa and northern wheatgrass), soil respiration, bacterial growth (ECHA biomonitors), total heterotrophic bacteria, nematode survival, earthworm survival, algal growth inhibition, and bacterial luminescence. Tests were done on both water and methanol extracts of the soils. These solvents also permitted resolution of the presence and availability of contaminants with different physical and chemical properties.

The soil samples spiked with HD did not have a strong toxicological impact on the microbial, plant or invertebrate species tested. The most sensitive endpoint noted was earthworm avoidance with a no effect concentration of 160 mg/kg. Mustard-lewisite applied to soils was highly toxic to all trophic levels tested, for both direct soil exposure tests, and aqueous and methanol extracts. The most sensitive endpoint was root elongation for the lettuce with a no effect concentration of 0.067 mg/kg. The results indicated that the soil health index test battery would provide a valuable tool for detection of agent-contaminated soils, and suggest that low levels of soil freshly contaminated with HL would pose a significant risk to soil-dependent receptors.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Appendices

1.0 INTRODUCTION

Chemical warfare agents, specifically mustard (HD) and mustard-lewisite (HL) mixtures have been used and stored in defence research and training establishments in Canada and abroad. Closure and decommissioning of these sites may involve the assessment of impacts of contaminants to both human and ecological receptors under the current or intended future land use. Assessment of ecological impact has been primarily based on compliance with chemical criteria set forth by various regulatory bodies. Most often, these criteria are based on extremely conservative estimates of risk to human health, or, criteria for many contaminants simply do not exist. For example, the NATO acceptable soil concentration for HD of 1 mg/kg is based on the protection of human health. Since effects of contaminants to ecological receptors are often not based on experimentally derived data, a lack of confidence in assessment findings can result.

DRES contracted Golder Associates Ltd. to determine the threshold for toxic effects for HD exposure to ecological organisms. Golder Associates retained HydroQual Laboratories Ltd. to develop and conduct a battery of laboratory tests on soil-dependent organisms (microorganisms, invertebrates and plants) using HD.

2.0 SCOPE OF WORK

The original scope of work for this project included:

1. Test MicroTox using HD to establish that this bacteria responds to HD;

2. Conduct tests on soil microbes, invertebrates and plants to determine the contaminant threshold for soil-dependent receptors.

Three significant changes to the original proposed scope of work occurred:

1. Due to regulatory restrictions on the transport, use and storage of HD and chemical warfare agents in general, toxicity testing was performed at DRES. All bioassays were conducted by HydroQual personnel at the DRES facility.

2. Chemical analyses were originally to be performed by a commercial laboratory. However, due to the restrictions noted above and budget constraints, chemical

analyses were conducted by DRES scientists. Results were not available for this report.

3. Finally, in addition to HD, a HL mixture was added to the testing protocol. The mixture was added due to its common occurrence at chemical agent contaminated sites abroad.

3.0 BACKGROUND

As part of the Ecological Risk Assessment at Defence Research Establishment Suffield (DRES) (Golder, 1997), a battery of toxicity tests was used to test the toxicity of soil samples to soil-dependent receptors including plants, invertebrates and bacteria. The approach used to the risk assessment was based on the assumption that soil-dependent receptors would be the most sensitive receptors to contaminants in soil. If so, clean up measures based on results for soil-dependent receptors would be protective of all receptors and could therefore be used to guide the remediation of the sites.

Although the soil-dependent receptors were known to be sensitive to conventional contaminants (e.g. metals), the toxicity of HD-contaminated soil to soil-dependent organisms was not known. In order to determine the threshold for toxicity of HD to the soil-dependent organisms tested, DRES provided a HD-spiked soil sample to establish a standard curve. The soil was spiked at a nominal concentration of 200 mg/kg – a concentration expected to generate a toxic response in most, if not all of the tests. However, the spiked-soil did not elicit a toxic response in some soil-dependent receptors, although a mild toxic response was observed in others (Table 1). Due to the scoring procedure used to incorporate the results of the toxicity testing into the risk assessment, overall, the response of the spiked soil was not significantly different than background soil samples.

The apparent high toxicity threshold to HD for soil-dependent receptors was unexpected, based on the NATO soil guideline. The implications for an apparent high toxicity threshold for soil-dependent receptors exposed to HD for the ERA were limited however, since although HD was detected on the DRES Experimental Proving Ground (EPG) in the past, HD was not detected in the DRES ERA. Several soil samples that exhibited strong toxicity were found to be contaminated with sulphur and a number of HD breakdown products. However, a low soil pH, most likely caused by the biodegradation

of HD and related compounds, was the most consistent potential causal factor associated with a strong toxic response. For the contaminants that were detected on the EPG, the soil-dependent organisms were the most sensitive receptors.

There are several possible explanations for the observed high threshold for soil-dependent receptors exposed to HD, three of which are discussed below.

1. The physical/chemical properties of HD may result in low bioavailability due either to a low water solubility or to adsorption to soil. The tests in the ERA that resulted in no significant toxicity were tests based on aqueous extracts. Since HD has a low solubility, it is possible that very little of the parent compound was present in the aqueous extract. The tests in which toxicity was observed were methanol extracts or direct tests on the soil. However, even the results for the direct tests of spiked soil did not show a strong toxic response.

2. HD also rapidly hydrolyzes when dissolved in water. It is possible that little of the parent compound was present in the aqueous extract that was used to test toxicity due to rapid hydrolysis.

3. Lack of homogeneity of the soil sample used for testing may have resulted in reduced exposure to soil-dependent receptors. However, the method DRES used to prepare the soil, which involved dissolving the mustard in hexane, treating soil with the mustard in hexane solution, and removing the hexane under vaccuum, would likely have achieved the best possible results.

4.0 PURPOSE

The purpose of the proposed work is:

1. To determine the threshold for soil-dependent organisms to HD and HL in soil and compare the threshold to that of other receptors.

This work is part of a larger study to develop a soil toxicity screening method, which would provide an efficient, inexpensive method for determining whether agent-contaminated soil is present at a site.

5.0 METHODS

Tests were performed either by direct exposure to soils without further processing such as drying or sieving, or by exposure to soil extracts using both water and methanol (4:1 ratio of solvent to soil). The use of two solvents permitted differential extraction of potential contaminants based on their physical and chemical properties. Methanol extracts were tested at a level below the effect level for methanol to the test organism (NOEC or no observed effect concentration, 0.1 to 5% dilution depending on test species).

The tests included:

- Microbes
  - bacterial luminescence
  - bacterial growth (ECHA)
  - total heterotrophic bacteria
- Plants
  - root elongation
  - seedling emergence
  - algal growth inhibition
- Invertebrates
  - nematode survival
  - worm survival
- Community Processes
  - soil respiration

This battery of screening test methods was developed by HydroQual Laboratories Ltd. for assessing soil health (Soil Health Index). Tests were selected to provide a range of acute and sublethal endpoints for major trophic levels in a soil environment, and to provide a mix of population and ecosystem endpoints. Collectively, the results provide insight on

the overall health and condition of the soil ecosystem and indicate potential for toxicological impact on soil communities.

Soil health is assessed in terms of abiotic and biotic properties and how these relate to the existing ecological state and future potential. Abiotic factors included physical and chemical conditions such as soil pH, electrical conductivity (salts), particle size distribution (sand, silt, clay), colour and odour. The abiotic characteristics defines areas which can support life forms and potentially, a viable soil ecosystem. Biotic factors measured include an assessment of indigenous bacterial and fungal populations, measurement of soil respiration (with and without augmentation), assessment of the potential to support growth of microbes, plants and invertebrates, and analysis for the presence of genotoxic compounds.

The species included in the test battery are representative of major trophic levels in soil systems. Plants and microbes convert chemical energy and light (plants) into biomass, and they also serve as primary food sources for soil invertebrates. The organic matter produced by plants also plays a crucial role in the physical structure and properties of soils. Invertebrates consume detritus, microbes and plants, and further form a critical link to higher level soil fauna and other predators. These invertebrates come into intimate contact with the soil and soil-bound contaminants.

The rationale for a test battery is that different species have different sensitivities to different compounds and conditions. Hence, effects are less likely to be missed with a test battery. This approach also permits resolution of the sensitivity of ecosystem components to different contaminants and conditions. This information can then be used to assess ecological risk, map for areas of concern for potential ecological impacts, as well as to select and evaluate remedial or management options.

5.1 Chemical Descriptions

All test chemicals used in this assessment were provided by DRES. Technical grade distilled HD (Standard NATO agreement code: HD) used in this study is composed of bis (2-chloroethyl) sulfide, molecular formula $Cl(CH_2)_2S(CH_2)_2Cl$. It is an amber brown liquid used as a blistering agent, and is among the most commonly listed military

casualty agents. The pure chemical is slightly soluble in cold water and soluble in most organic solvents. The parent compound rapidly hydrolyses in distilled water (within 17 minutes) forming hydrochloric acid and thiodiglycol. Volatility is 75 mg/m^3 as a solid at 0°C, and 610 mg/m^3 as a liquid at 20°C. The vapor density is 5.4 times heavier than air. The material is sometimes observed to remain as persistent micro encapsulated crystals in soil (JM McAndless, personal communication). The compound is decontaminated by strong oxidizing agents and by alkaline hydrolysis.

Mustard-lewisite mix, designated as HL by the Standard NATO agreement code, is composed of a mixture of HD at a rate of 37-50%, and lewisite (Standard NATO agreement code: L) at a rate of 50%-63%. The active ingredient in lewisite is dichloro (2-chlorovinyl) arsine, chemical formula: ClCH:CHAsCl_2. Lewisite rapidly forms a heavy (7 times heavier than air) vapour which rapidly hydrolyses to hydrochloric acid and chlorovinylarsenious oxide (mildly active). Lewisite has a low water solubility, but unlike HD, does not hydrolyze in water and remains relatively persistent. Decontamination is effective with bleaches and oxidizers, and strong alkalies such as sodium hydroxide.

5.2 Soil Preparation

Two soils, artificial soil prepared from standardized recipes, and field collected clean soil, were used for this study. The purpose of using the artificial soil was to provide standardized results which could be directly compared to the known toxicity of other compounds determined in this standard soil, and to indirectly evaluate the fate and behavior of HD in soil by testing in two differing soil types and comparing toxicity. Differences in results between the two soils would imply that the fate of HD in soil, and therefore the toxicity, was influenced by soil components (e.g. differences in soil pH, organic, sand or clay content effects availability or loss of the parent compound).

Artificial soil was prepared according to standard recipes published by Greene et. al. (1989) and accepted by a number of standards organizing bodies (ASTM 1996 a, 1996b; OECD 1993). The soil was prepared by addition of 80% silica sand, 10% 5-mm sieved peat moss and 20% kaolinite clay. No attempt was made to increase the pH of the soil, which is acidic in nature (pH 4) as a result of the peat moss component. The highly

acidic soil would provide a contrast to the neutral field soil, allowing potential inferences on soil behavior of the parent compounds. The pH is within the laboratory-established known tolerance range of organisms tested. The soil was mixed by tumbling for 24 hours. A single batch of soil was prepared for the entire study (chemical analysis appended).

The field soil used in this study was collected by DRES personnel from a clean site on the EPG. The soil was described as predominantly Chernozem, with an accumulation of organic matter, a brown colour, granular structure, neutral pH and low water holding capacity (Kjseargaard, 1973).

Both artificial and field soils were fortified with HD and HL by DRES scientists one day before initial toxicity testing was done. Soils used for controls and dilution were treated in the same manner as the chemically fortified soils.

All work involved in spiking soil samples with chemical warfare agents and subjecting soil samples to toxicity testing was carried out in a fume hood in the Canadian National Single Small Scale Synthesis Facility (CNSSSF), Chemical Containment Area, Defence Research Establishment Suffield.

The following agents were employed in the study of the soil toxicity method:

1. Mustard (HD, bis(2-chloroethyl) sulphide, CAS 505-60-2)

2. Lewisite (L, 2-chlorovinyl dichloroarsine, CAS 541-25-3) mixed equi-volume with HD, to give agent HL.

5.2.1 Method for Preparing Stock Solutions Containing Agent

In order to determine agent bioavailability, two stock solutions, one containing HD in 5% methanol in water (v/v), the other containing HL in 5% methanol in water were prepared. The 5% methanol solutions were then subjected to Microtox toxicity tests.

A HD stock solution was prepared by DRES by first dissolving in reagent grade methanol (0.1023g in 5 mL) and then taking to 100 mL volume with deionized water. The agent

appeared to dissolve completely in the methanol. Fine drops of the agent appeared to come out of solution as the water was added. On standing for several hours, the droplets appeared to re-dissolve. Assuming no hydrolysis occurs, the theoretical concentration of HD in the 5% methanol HD stock solution was 1.023 mg/mL based on the weight of HD added.

HD (0.0514 g) was weighed into a 100 mL volumetric flask, followed by 0.0632 g of munitions-grade Lewisite (contains approximately 85% lewisite by weight). Methanol (5 mL) was then added to the flask using a pipette. Both agents appeared to go into solution as the methanol was added. De-ionized water (95 mL) was then added to the mark in the volumetric flask. Fine drops of agent (probably HD) came out of solution as the water was added, then slowly re-dissolved over a period of several minutes. Based on the weights of agents added, and taking into account the purity of munitions-grade Lewisite, the agent composition of the final HL stock solution is as follows: H:- 0.514 mg/mL (49%); L:- 0.537 mg/mL (51%) for a total HL of 1.051 mg/mL.

5.2.2 Method for Preparing Agent-Contaminated Soil

Two stock solutions were prepared by DRES scientists, one containing HD dissolved in hexane and the other containing HL dissolved in hexane were prepared (HD 0.06338 g/mL and lewisite 0.09725 g/mL).

Artificial soil, supplied by Hydroqual Laboratories, and DRES EPG soil were spiked with HD in similar fashion to yield soil contaminated with the agent at a concentration of approximately 1000 mg/kg soil. Prior to commencing spiking experiments, each type of soil was weighed into a tared 4L glass jar until 3 kg of soil had been added. The glass jar was then marked at the fill level represented by this added weight of soil. For each spiking experiment a new, clean glass jar was utilized. Approximately 500 g of soil was added to the jar followed by addition of 50 mL of the stock hexane solution containing HD. The soil was mixed by rolling the sealed glass jar on a motor-driven mechanical roller for 30 minutes. When the headspace of the jar was surveyed with a Chemical Agent Monitor (CAM) immediately following mixing, a positive (4-6 bars H mode) response was obtained, indicating the presence of HD vapour. The open jar was then placed on a hotplate set at 50 °C and heated for one hour to remove the hexane by

evaporation. After this, a second headspace survey with CAM produced a positive, 4-bar response, indicating the presence of HD vapour. Further soil, which had previously been treated with hexane (see below), was then added to the jar to the appropriate fill level. The jar was then rolled for 3-4 hours, with occasional manual shaking, to thoroughly mix the agent-contaminated soil. Following mixing, a headspace survey of the jar did not produce a CAM response.

On a weight basis, the artificial and DRES EPG soils were thus contaminated at a HD concentration of 1231 mg/kg of soil.

For the HL soils, approximately 500 g of soil was added to the jar followed by addition of 25 mL of the stock hexane solution containing HD and 25 mL of the stock hexane solution containing lewisite. The soil was mixed by rolling the sealed glass jar on a motor-driven mechanical roller for 30 minutes. When the headspace of the jar was surveyed with a Chemical Agent Monitor (CAM) immediately following mixing, a positive (4-6 bars) response was obtained in the H-mode, indicating the presence of vesicant agent vapour. The open jar was then placed on a hotplate set at 50 °C and heated for one hour to remove the hexane by evaporation. After this, a second headspace survey with CAM produced a positive, 4-bar response, indicating the presence of vesicant agent vapour. Soil which had previously been treated with hexane (see below), was then added to the jar to the appropriate fill level. The jar was then rolled for 3-4 hours, with occasional manual shaking, to thoroughly mix the agent-contaminated soil. Following mixing, a headspace survey of the jar did not produce a CAM response.

On a weight basis, corrected for purity of munitions-grade lewisite, the artificial and DRES EPG soils were thus contaminated as follows:

HL concentration: 1380 mg/kg of soil, consisting of;

41% H:- 570 mg/kg of soil, and

59% L:- 810 mg/kg of soil

5.2.3 Preparation of Hexane-Treated Control Soil Samples

Control soil samples were prepared in order to note any effects on the toxicity tests of using hexane as the solvent to spike soils with agents. These control soils were used to dilute the agent-spiked soil to the appropriate contamination concentration, as described above. The Artificial Soil and DRES EPG Soil were treated in similar fashion as follows:

Approximately 7-8 kg of soil was placed in a 10 L plastic carboy. To this, 350 mL of hexane (Burdick & Jackson GC capillary column Grade) was added. The carboy was then rolled for 7-8 hours on a motor-driven mechanical roller to thoroughly mix the soil. A second batch of the same soil type was prepared in similar fashion. The two batches were combined in a 20 L plastic pail and the pail and contents were then placed in a forced air oven set at 50 °C. After heating the soil for one hour to remove the hexane, the soil was spread out into a large metal tray covered with a plastic liner and allowed to air overnight before being stored in the 20 L pail for use in the soil spiking experiments.

Soil treatment for use in toxicity testing were prepared to give the following nominal concentrations: 26, 64, 160, 400 and 1000 mg/kg. This concentration series was chosen based on the results of the Microtox test results for the stock solutions of pure compounds. The 1000 mg/kg soil was serially diluted by transferring 1 kg of the highest treatment to 1.5 kg of hexane-treated soil and mixed by tumbling end over end (400 mg/kg). The serial dilution was continued until all treatments were prepared.

5.2.4 Preparation of Test Treatments

Stock solutions of both chemicals were prepared on the same day as soil spiking took place for the purpose of assessment of direct toxicity of the pure compound to selected species. The chemical stock solutions were tested for bacterial luminescence by diluting the 1000 mg/L stocks with deionized water. Results are reported as HD or HL in mg/L, nominal concentrations. Each test included an untreated soil control (artificial or field soil, no manipulation) and a hexane-treated soil control.

Soils were stored dry under ambient laboratory conditions until test initiation. Soils were distributed to various test vessels on a whole weight basis as described in the following sections for individual test methods. Soil extracts were prepared for the four soils using

deionized water and reagent-grade methanol. Extracts were prepared by transferring 150 g of each of the four 1000 mg/kg soils to a 1 L plastic bottle and adding 600 mL of the appropriate solvent. The extracts were manually shaken for two minutes, then allowed to settle for approximately 18 hours. The clarified extract was removed from the extraction bottle and transferred to a clean container. Extracts were stored under ambient laboratory conditions until tested.

Test treatment for the deionized water extracts were prepared to obtain the following concentrations: 2.6%, 6.4%, 16%, 40%, and 100%. The methanol test treatments were 0.026%, 0.064%, 0.16%, 0.40% and 1.0%. The highest dilution of methanol that could be tested was 1.0%, due to the inherent toxicity of methanol to the test species. Treatments were prepared by serially diluting the 100% extracts by a factor of 0.4 using deionized water, or in the case of the methanol extracts, using a 1% methanol in deionized water solution, to maintain constant methanol concentrations in all treatments. The controls used for extraction tests included a laboratory control consisting of deionized water or 1% methanol, and a soil control (aqueous or methanol extract of hexane-treated control soil). Concentrations are reported as percent dilution of the extract. Lower test dilutions were included where appropriate to obtain a dose-response relationship.

The following sections describe test methods for soil exposure tests, including seed emergence, earthworm survival, ECHA dipsticks and soil respiration, and the extraction tests, including root elongation, bacterial luminescence, heterotrophic bacteria, algae growth inhibition and nematode survival. Test procedures are based on available accepted standard methods where available, and references are provided in Section 10.

5.3 Microbial Tests

Microbes are an integral component of soil systems. They play vital roles in the degradation of organic mater, the cycling of organic nutrients and metals, and serve as an important food source for many invertebrates. The microbial tests included bacterial luminescence (Microtox test), bacterial growth (ECHA dipsticks), and enumeration of soil bacteria.

The bacterial luminescence test is based on light output by the marine bacterium *Vibrio fisheri* (Environment Canada, 1992). The bacterium is exposed to the sample (extract) and light levels are measured at 15 minutes. Substances that are toxic or stressful will reduce light output. The test was included in the SHI battery since it is rapid, requires small sample volumes and is relatively sensitive to a variety of contaminants. Therefore, it is ideal for screening of large numbers of samples.

The sensitivity of *Vibrio fischeri* to HD and HL was evaluated by testing the 1000 mg/L stock solutions immediately after preparation, to reduce loss of the parent compounds. The bacterial luminescence test was done first in the test battery, in order to establish relative sensitivities of test organisms for determining test concentrations for other species. Additionally, the stock solution was retested after 24 hours, and again later, to establish if loss of toxicity could provide evidence of loss of the parent compound by hydrolysis or other means.

The stock solutions, aqueous and methanol extracts were tested by diluting the 100% solutions to appropriate concentrations with deionized water which would elicit a no-effect and effect response. Test solutions were osmotically adjusted, then bacteria were exposed for fifteen minutes. Light readings of the exposed bacteria in each test concentration and controls were measured using the Microtox Model 500 Unit. Results are presented as light inhibition relative to controls in mg/L nominal concentration for stock solutions and as percent dilution of the extracts.

Density of total heterotrophic bacteria in the HD and HL field soil samples were enumerated by a mean probable number method, or MPN (Carter, 1993). The aqueous extract was diluted to 10^{-2}, and was further diluted in a 96 well microplate (10^{-3} to 10^{-8}) using Peptone Yeast extract media. There were four replicates per sample. Growth was scored by the presence of turbidity in the wells after 5 days of incubation at 23°C. The bacterial density was determined from the number of positive wells in each dilution, based on probable number tables. The density of indigenous heterotrophic bacteria in the field soil was compared to the fortified soil samples in order to determine if HD compounds had a toxic effect on indigenous populations. Artificial soils were not included in the test since interest is in indigenous field soil populations, and artificial soil is inherently sterile.

5.4 Plant Tests

The plant tests included seedling emergence, root elongation and algal growth inhibition. The first test was done by direct exposure to the soil. The other two tests were done on the water and methanol extracts.

The seedling emergence test method was based on the procedure developed by Green et al. (1989) and presented by standards organizations (OECD 1993, ASTM 1996b). The test species included lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*) and northern wheatgrass (*Agropyron dasystachyum*) selected in order to provide a representative commercial, agricultural and native test species. The three species selected were based on recommendations for species sensitivity, time to test endpoint and germination success (Stephenson et al., 1997b). Seeds were pretreated with a 0.5% sodium hypochlorite solution, followed by a deionized water rinse, then air dried immediately before use in tests. Three replicates were set up for each of the three species.

The tests were conducted in plastic Petri dishes containing 30 g of each treated soil. Large rocks and other debris were removed from the field soil by hand. Twenty seeds were placed on the surface of the soil and covered with a sand cap (30 g of washed silica sand). The soils and sand cover were hydrated with deionized water to achieve 80% of the soil's water holding capacity (15 mL for artificial soil and 9 mL for field soil). The dishes were then sealed with Parafilm and incubated at 23°C in the dark. After 48 hours, the dishes were exposed to ambient laboratory lighting (16 hour light and 8 hour dark photoperiod) on a benchtop which also received natural sunlight.

The tests were scored for seed emergence on Day 6. Shoots extending above the sand cap were considered emerged. All results are expressed as the percent emerged, relative to the rate of emergence in the hexane-treated soil control.

Root elongation tests were conducted with the same test species, on the aqueous and methanol extract dilutions following the procedure of Greene et al. (1989). Ten seeds were exposed on Whatman No. 3 filter paper in a 10 cm plastic Petri dish (1 replicate for each species). The paper was moistened with 4 mL of the appropriate extract treatment. The dishes were capped with lids and sealed with Parafilm. Root lengths were scored

after a 6 day incubation period in darkness at ambient temperature. Seeds with root tips emerging or with a split seed coat were considered germinated. Lettuce and alfalfa root lengths (hypocotyl) were measured from the root tip to the base of the shoot (epicotyl). The transition between the root and shoot of lettuce seeds is clearly defined by a sharp bend. Wheatgrass root lengths were measured from the root tip to the seed coat. The results were expressed as a mean percent of the hexane-treated soil control extract.

The algal growth inhibition test was done with the unicellular green alga *Selenastrum capricornutum* (Environment Canada, 1992). This species is common to many freshwater lakes and ponds in North America. The test was performed on the water and methanol extracts. Nutrients required for miniminal algal growth were added directly to each treatment followed by an inoculum of an in-house algal culture in an exponential growth phase, to a final concentration of approximately 10,000 cells/mL. The alga was exposed to the sample treatments in 96 well microplates, replicated three times for each treatment.

Effects on growth were measured after a 3 day exposure period, under continuous light (4000 lux) at ambient temperature. Any substance or condition that is stressful will inhibit or retard growth, resulting in a lower final cell density. Increases in final cell densities over the controls may result from the presence of nutrients or other essential trace substances in the samples. The observed results were based on optical density measurements, calculated as percent density compared to the laboratory control density. Results are expressed as percent growth inhibition, relative to the control.

5.5 Invertebrate Tests

The invertebrate test species included the nematode *Panagrellus redivivus* and the earthworm *Eisenia fetida*. Nematodes and earthworms play a vital role in soil ecosystems as both consumers of detritus and microbes, and as food for other invertebrates and predators.

Nematodes were exposed to several dilutions of the aqueous and methanol in 96 well microplates containing four replicates per sample. Mortality was scored after a five day

exposure period at 23 ± 1°C. Results are expressed as % survival relative to numbers exposed.

The earthworm survival test is a short-term acute lethality test (Greene et al., 1989). Two hundred grams of each soil treatment were distributed to 250 mL plastic cups, and hydrated with deionized water to 80% of the soil's water holding capacity (100 mL for artificial soil, 40 mL for field soil). Untreated and hexane-treated soils were included as controls. Each treatment was replicated once only, since our experience has shown that worm sensitivity to toxic compounds is usually displayed as an all or nothing effect, and therefore, replication increases test setup effort without increasing confidence in test results. Ten mature worms (*Eisinia fetida*) were introduced to each test chamber, which were covered with a plastic lid, and incubated at 23°C under ambient laboratory lighting. After 7 days of exposure, the number of live worms was scored in each cup. Observations were also made on the distribution of worms within the test chamber; avoidance, a sensitive sublethal endpoint, was indicated by surviving worms clumped on the surface with no penetration of the soil.

5.6 Soil Community Tests

Soil respiration, or the production of carbon dioxide, is a gross measure of total biological activity or community respiration. High levels of soil respiration are an indication of a healthy soil ecosystem. High rates should parallel large populations of microbes with a good organic food source and indicate that the physical and chemical conditions are not harmful. It should be noted that some soils will bind or release carbon dioxide, and in such cases the measurement of oxygen provides a better indication of biological activity. However, atmospheric carbon dioxide levels are much lower than oxygen (375 ppm compared to 20.8%). For this reason, it is easier to detect smaller changes in carbon dioxide levels over shorter time periods.

Ten grams of field soil was placed into a 20 mL headspace vial and moistened with deionized water. The vial was capped with a teflon septum, which was held in place with a crimped ring. Soil respiration was determined for field soil treatments only, at the low, medium and high concentrations due to time limitations. Additionally, a replicate vial of each treatment was augmented with D-glucose at 1000 mg/kg to provide a carbon source

for bacterial growth. This was included to help distinguish negative effects from poor bacterial populations due to poor soil nutrient abundance.

Headspace carbon dioxide levels were measured after seven days of incubation at 23°C. Carbon dioxide was measured on a Hewlett Packard 5700A gas chromatograph, equipped with a thermal conductivity detector and a 60/80 Carboxen 1000 column (hydrogen carrier gas 30 mL/min; oven temperature of 200°C; retention time for CO_2 of 2.5 min.). The results were expressed as the fold increase in headspace carbon dioxide levels relative to a control (vials without soil) for unaugmented and augmented soils. The ratio of unaugmented:augmented respiration was also calculated. High values in both untreated and amended soils indicate a healthy soil community with adequate nutrient and organic content to support a diverse population. Soils with low values but higher levels with organic amendment indicate that the soil may be nutrient deprived. Low values for both unaugmented and augmented soils may indicate poor community health due to toxic conditions as a result of presence of contaminants, or other excessive physical or chemical conditions adverse to support of a healthy community.

5.7 Quality Assurance

A number of quality assurance procedures were incorporated into each test. These procedures were in addition to those routinely followed as part of HydroQual's Quality Assurance Plan. Specific procedures included the use of positive and negative controls and replicates. Reference toxicants are used as positive controls to assess the health, condition and relative historical sensitivity of the test populations. The test result or response must fall within predefined limits, based on historical values. Values outside the limits can indicate a change in the sensitivity of the organism or change in test conditions.

Zinc sulfate, 2-chloroacetamide and sodium chloride were used as positive controls for seedling emergence and root elongation (lettuce, alfalfa and northern wheatgrass), algal growth inhibition, bacterial luminescence, nematode survival, earthworm avoidance and soil respiration. These values are expressed as the concentration of toxicant required to give a 50% change in the response measured, relative to controls (IC50, inhibitory concentration; EC50, effective concentration, LC50, lethal concentration). Reference

toxicants can be used to interpret results obtained at different times and amongst different test conditions or facilities. They serve as a valuable benchmark or reference point for comparative and interpretative purposes. Additionally, positive control results provide an indication of relative sensitivities of the test organisms to major toxicant classes, and can provide information on cause of toxicity in test samples based on trends in responses relative to each species.

A negative control is a treatment that does not have an effect on the test organism (a baseline or laboratory control). The response in the negative controls must not exceed a predefined level for a test to be considered valid. Negative controls were included for all test procedures to indicate the optimal response to which sample results are compared for relative test endpoints such as root length, soil respiration, bacterial luminescence light output and worm avoidance.

The last element in the quality system is the reporting of data. All data were independently reviewed and verified by the Quality Assurance Unit.

6.0 RESULTS AND DISCUSSION

The results and discussion which follows provides a short review of findings for each trophic level, followed by a general discussion and summary of results. Test data results are presented in Tables 2 to 11.

Aqueous and methanol extracts were prepared on each sample as previously described. The 4:1 liquid to solid ratio was used as an approximation of worst case leaching conditions in the field. Also, separation of the liquid and solid phases becomes problematic at lower liquid to solids ratios. Methanol is used to remove more hydrophobic substances from the solid phase (primarily organic compounds). Although not directly applicable as a representation of real leaching conditions, it provides information on contaminant type, and can indicate impact potential from long-term exposure of organisms to hydrophobic contaminants by direct physical contact to contaminated soil and pore water (chronic toxicity, bioaccumulation and biomagnification). The water extract is more representative of materials that are readily

leached from the solid phase and are therefore more available to soil flora and fauna (substances that could end up in groundwater and surface water).

The aqueous extracts of the HD and HL artificial and field soils were measured for pH and conductivity (Table 2). The physical characteristics of the soils as measured are considered within acceptable limits to support most terrestrial life. No other unusual conditions were noted.

6.1 Microbial Tests

6.1.1 Bacterial Luminescence

The microbial tests included bacterial luminescence (Microtox), bacterial growth (ECHA), and bacterial enumeration.

An attempt was made to expose *Vibrio fischeri* directly to HD so that exposure to products of rapid hydrolysis could be avoided, by adding the product directly to test vessels containing bacteria. However, 100% toxicity was observed in the lowest volume of HD which could be measured for the test vessels used (1µl of HD added to 1 mL = 1.27 mg/mL). The result suggests that the parent compound is toxic to 1270 mg/L. A modified test system (larger volumes for bacterial exposure) would be required to evaluate direct exposure to lower concentrations of HD.

The toxicity of pure HD and HL was evaluated by testing stock solutions (5% methanol) of the products with bacterial luminescence (Table 3). Pure compound was added to a 100 mL volumetric flask, followed by 5 mL methanol. Both products dissolved completely in the methanol. However, both products appeared immiscible once the solutions were brought to volume with deionized water, forming small droplets of product distributed within the solution. The pH of the stock solutions were 2.2 for the 1000 mg/L HD, and 1.8 for 1000 mg/L HL. Stock solutions were initially tested immediately after preparation to minimize loss of compounds by hydrolysis.

Despite apparent insolubility of the products in water, both HD and HL were toxic to *Vibrio fischeri*. HD toxicity was relatively moderate, with an IC50 of 100 mg/L shortly after stock preparation (10% of the stock solution). Adjustment of pH of the solution to

6.6 resulted in an IC50 of 58 mg/L, which confirmed that the apparent toxicity of the stock was due to exposure to HD rather than the acidic pH of the solution. Toxicity of HD appeared to increase over time, as indicated by IC50 results when the stock was tested 24 hours and 8 days after the initial stock preparation. This trend would require confirmation since insufficient data points were measured over time for this study.

The observed toxicity was unexpected since previous knowledge of the behavior of HD in aqueous solutions indicated a rapid hydrolysis (within 17 minutes) in distilled water at 25°C to hydrochloric acid and thiodiglycol (J.A.F. Compton, 1987). Therefore, either toxicity was due to exposure to HD, and hydrolysis is not as rapid as previously thought, or that toxicity was due to exposure to the hydrolysis product, thiodiglycol, or other breakdown products. If the latter is the case, then the hydrolysis product appears to be stable. The cause for toxicity could be evaluated by testing known breakdown products as pure compounds, over a period of time to monitor behavior. Additionally, the toxicity of pure HD could be evaluated by exposure of the bacteria directly to HD by introducing the whole material to test vessels containing bacteria. In this manner, bacteria are exposed to both the parent compound and hydrolysis products that are formed within minutes of exposure to an aqueous solution. The results could then be compared to the toxicity of known breakdown products. An alternative test would be to force hydrolysis of the parent compound by strong alkaline or oxidizing conditions, with confirmation of products by analysis, then testing in comparison to the acidic HD stock solution.

Relative to HD, the aqueous stock solution of HL mixture was highly toxic to *Vibrio fischeri*, with an IC50 of 0.027 mg/L (greater than 3000 times more toxic than HD) (Table 3). Adjustment of pH of the stock solution had no affect on toxicity. The results were consistent over time, as measured at 1 day and 8 days after stock preparation, indicating a relatively stable compound.

The HL stock was prepared as a 50% mixture each of HD and lewisite (L). The actual composition of the stock solution was 514 mg/L HD and 537 mg/L lewisite. Therefore, assuming toxicity of the solution was due to lewisite, the IC50 of lewisite to bacterial luminescence is 0.014 mg/L (IC50 of HL 0.027 mg/L * 51% as lewisite).

The stability of lewisite, as indicated by the relatively stable bacterial luminescence results over 8 days, was expected. Lewisite is known to have a low water solubility, and a relatively slow hydrolysis rate in aqueous solutions. Hydrolysis of the product to hydrochloric acid and chlorovinylarsenious oxide occurs rapidly in the vapour phase, or under strong alkaline or oxiding conditions. Like HD, the long-term stability of toxicity of the parent lewisite compound, and the effects of breakdown products is unknown, and warrants investigation.

The toxicity of metallic arsenic and arsenic salts has been previously investigated (CCME, 1993). Arsenic salt, as KH_2AsO_4 has a Microtox IC50 of 630 µg As/L, indicating that the organometallic compound, lewisite, has a greater degree of toxicity than the metallic compound alone.

Bacterial luminescence tests were conducted on the water and methanol (5%) extracts of the field and artificial soil samples spiked with HD or HL. Exposure of *Vibrio fischeri* to aqueous extractions of 1000 mg/kg HD in artificial and field soils in general did not markedly reduce light output (Table 4). HD in artificial soil resulted in an IC50 of 41% of the extract, while field soil was > 91%. The difference between the artificial and field soil is likely due to toxicity of pH in the artificial soil to *Vibrio fischeri* (pH 3.2 in artificial soil extracts compared to pH of 6.1 for field soil extracts). The tolerance limit for *Vibrio fischeri* is about pH 5.5. Due to time constraints, no attempt was made to test with pH adjustment of the extracts.

Methanol extracts of HD in the soils proved to be more efficient than aqueous extraction, as indicated by toxicity to bacterial luminescence. The IC50 of HD in artificial soil was 4.9%, and for field soil, 5.6%. These levels are approaching concentrations of methanol toxic to *Vibrio fischeri*. Therefore, slightly lower degrees of toxicity from lower initial concentrations, biodegradation or volatile losses could not be detected for HD in a methanol extract, since a 5% solution of methanol is required to prevent toxicity of methanol to the bacteria.

Based on the toxicity of the pure compound in a stock solution, the recovery of HD in artificial and field soils is relatively poor. The extraction, a 4:1 ratio of a 1000 mg/kg nominal HD concentration, would result in a concentration of 250 mg/L HD present in

the extract, if recovery was complete. This concentration would result in an IC50 of 7.6% of the extract, based on an IC50 of HD of 19 mg/L, measured on Day 8 post-stock preparation. The lack of sensitivity of the aqueous extractions of HD in comparison to the expected toxicity suggests that: a) HD has degraded in soil to nontoxic compounds; b) HD has adsorbed to soil components and is not bioavailable; or c) HD solubility in aqueous solutions is too low to prevent efficient extraction. HD is known to have low availability in soil due to formation of microencapsulated crystals within the soil matrix, so that poor aqueous extraction may be due to low bioavailability.

The mass/toxicity balance of HD in methanol extracts indicated that HD is likely extracted completely from soils with methanol. Toxicity of methanol extracts in both soils was near that expected based on soil concentrations. Since concentrations are near the methanol toxicity limit, fortification of soil to higher concentrations than the 1000 mg/kg concentration with subsequent extraction would be required to confirm this. Regardless, the methanol extractions indicate that toxicity of HD or it's degradation products measured in the stock solutions was present in soils, recovered in the methanol extract, indicating that losses due to volatilization or breakdown to nontoxic compounds had not occurred within the timeframe of testing done. Therefore, it is likely that HD either weakly adsorbs to soil components made unavailable to an aqueous extraction, or the aqueous solubility of HD in soil is too low to be recovered in an aqueous solution, but is available to be removed by methanol.

HL was recovered equally in both aqueous and methanol extracts. No differences were observed for soil type. The IC50s were as follows; aqueous and methanol extract in artificial soil was 0.014% and 0.011%, and in field soil, 0.016% and 0.019%, respectively. Mass balance of toxicity with soil concentrations for HL indicates a high level of extraction efficiency. Based on an IC50 of 0.038 mg/L (day 8 test) for HL, an extract would be expected to have an IC50 of 0.015%. This confirms that recovery of the toxic constituent was 100% for both a 4:1 aqueous or methanol extract. The efficiency of extraction is likely a reflection of chemical behavior and toxicity of lewisite alone.

The lewisite toxicity data indicate that Microtox testing of soil extracts could provide a powerful and sensitive tool for detection of HL in contaminated soils. Based on these conditions, the detection limit for extraction and toxicity for HL is estimated as

152 µg/kg contaminated soil (4:1 extraction, IC50 = 100% extract), or approximately 76 µg/kg for Lewisite. This assumes that extraction efficiency is not influenced by universal soil characteristics, that aging has no effect on recovery, and that recovery efficiency is not influenced by soil concentration (linear relationship of recovery with dose). These assumptions require verification. The effect of aging soils on extraction efficiency is unknown. Additionally, although field soil and artificial soil are two widely varying soil types in general soil characteristics, the effect of other soil types on fate and extraction efficiency of HL is not known. Finally, extraction efficiency may be related to dose; a low concentration of HL may not be recovered as well as the unrealistically high concentration of HL tested for this study.

6.1.2 Echa Biomonitors

ECHA biomonitors, like the bacterial luminescence test, measures toxicity to the test bacteria by exposure to soluble toxic compounds present in the sample. Each soil concentration was tested by preparing a 1:1 slurry with deionized water, then exposing the dipstick to the aqueous phase of the slurry after a few seconds of mixing (soil concentrations were 60, 120, 250). Therefore, the bacteria are exposed only to soluble, biologically available compounds in the soil sample.

Like the bacterial luminescence test, bacterial growth inhibition as measured by the ECHA dipsticks was not inhibited for test soils contaminated with HD (Table 5) to the highest test concentration. HL exposure, however, resulted in complete inhibition of bacterial growth to the lowest concentration tested, 26 mg/kg. The relative sensitivity of the ECHA dipstick compared to the bacterial luminescence test can not be determined without further testing to define the no-effect concentration. Since this test method is easily applied to field testing situations, the detection limit of the test would be worth determining for purposes of field screening for detection of lewisite.

6.1.3 Toxicity to Indigenous Bacteria

The bacterial counts (total heterotrophs) were done only in field soil at the highest concentration (1000 mg/kg). The control field soil had a moderate bacterial population density (MPN = 2300/g soil). In contrast, the 1000 mg/kg HL soil was completely sterile (MPN=0) indicating that HL is toxic to natural field microbial populations. This result is

ecologically significant, since loss of bacterial populations in soil has a negative impact on the diversity and function of the terrestrial ecosystem. Additionally, many organic contaminants are degraded by action of soil microbial populations. A loss of microbes in a contaminated soil may result in an increase in residence times of the contaminant, resulting in persistent contamination.

HD contaminated field soil was not toxic to indigenous bacterial populations. Interestingly, bacterial population density was increased significantly to 10^{10}/g. The reason for the dramatic increase in population is unknown, but may reflect breakdown products (e.g. high sulphate, fertilizer-type compounds) of HD providing a nutrient supply.

6.2 Plant Tests

RtThe results from the plant tests are reviewed in this section. This includes root elongation, seedling emergence, and inhibition of algal growth.

The seedling emergence test is reflective of the soil's potential to support plant life. The data obtained from the lettuce, northern wheatgrass and alfalfa seeds were generally quite consistent. Seed emergence was not severely impacted by exposure to HD, except in the highest test concentration (Table 6). Emergence was slightly reduced for all three species in artificial soil, and completely inhibited for northern wheatgrass in field soil. For both soils, all three species, growth in the 1000 mg/kg soil of emerged seeds reflected toxic effects; shoot height was markedly reduced relative to control shoot height, and no roots were present.

Seed emergence was strongly inhibited in both soils for all three plants species for HL. Emergence was inhibited generally in 64 mg/kg and greater, with sublethal effects such as reduced shoot height and lack of root development observed as low as 26 mg/kg, the lowest concentration tested. Lettuce appeared to be more sensitive than the other two species.

Root elongation, like seed emergence, was only slightly impacted by exposure to HD. Greater than 50% reduction in root length was observed only in the 100% aqueous extract of the 1000 mg/kg HD treatment in artificial soil. Similarly, methanol extracts were not

toxic to root development. This is not surprising, considering that the maximum concentration which could be tested was 1% of the extract (nominal concentration of 2.5 mg/L of HD in the extract, assuming 100% recovery)

HL was toxic to root development for all three species tested (Table 7). Sensitivities were remarkably similar among the three plant types, with lettuce slightly more sensitive than the other two species. There were no apparent differences between toxicity in either artificial or field soil, and endpoints were about the same whether water or methanol was used as the extraction solvent. Thresholds for toxicity for tests with field soils, aqueous extracts, ranged from a low of 0.067 mg/kg for lettuce to 6.4 mg/kg for alfalfa. Finally, relative sensitivities were similar to that observed with the bacterial luminescence tests.

Algal growth tests were conducted on the water extracts and at a maximum of 1% solution of the methanol extracts (Table 8). In all cases, the methanol and aqueous extracts of HD were not highly toxic to *Selenastrum*. Like the bacterial luminescence results, only moderate to slight inhibition was observed for aqueous extracts of HD. Small differences between toxicity of field and artificial soil extractions are likely due to the negative impact from acidic pH of artificial soil.

HL was highly toxic to algae, with little differences between artificial or field soil, and water or methanol extracts. The IC50 of HL ranged from 0.077 to 0.30%, for aqueous and methanol extracts, respectively. The NOEC from the field soil was 0.17 mg/kg for the aqueous extract and 0.26 mg/kg for the methanol extract. Although highly sensitive, *Selenastrum* is approximately 10 times less sensitive than the bacterial luminescence test. In general, relative sensitivities of algae and *Vibrio fishceri* to metals is opposite that displayed here; algae tend to be several orders of magnitude more sensitive to metals than *Vibrio fisheri* . This may again display the increase in toxicity of the organometallic compound compared to arsenic alone.

6.3 Invertebrate Tests

The earthworm test is a measure of toxicity of an invertebrate exposed directly to contaminated soil for seven days. Test endpoints are based on lethality. Earthworms also have chemoreceptors covering most of their body surfaces, and therefore, are often able

to avoid contaminated soils. Avoidance was included as an observation, indicated by lack of penetration and remaining on the soil surface.

Soils contaminated with HD were not lethal to earthworms over a 7 day period, up to 1000 mg/kg. However, avoidance of the contaminated soil was noted to 160 mg/kg and greater. This suggests that HD or toxic breakdown products are present and available in soil. Tests have been developed which can provide statistical endpoints of preferential avoidance of earthworms to soil contaminants. The earthworm avoidance test has been cited as having sensitivity to a number of contaminants to the degree causing acute lethality or reproductive effects in longer-term worm tests (Stephenson et. al, 1997). This is due to the worm's high density of chemoreceptors covering most of its body, allowing it to avoid adverse conditions. The observance of avoidance to 160 mg/kg provides the most sensitive endpoint to HD from the test battery used for this project.

HL was acutely lethal to earthworms exposed for seven days to concentration of 160 mg/kg and greater. Avoidance was noted to 26 mg/kg in the artificial soil, the lowest concentration tested. Lethality was slightly less sensitive to direct exposure of lewisite compared to the other direct soil exposure test - seed emergence. However, avoidance may be a more sensitive test endpoint, requiring further investigation.

The nematode test was designed, like the earthworm test, to represent invertebrate populations in the soil. Aqueous extracts, and a maximum of 1% of the methanol extracts were used for exposure of nematodes. Nematodes were not sensitive to HD or HL in methanol, and were only moderately effected in water extracts of HL. Therefore, lack of sensitivity of this species warrants removal from the test battery for detection of HD or HL in future work.

6.4 Soil Community Tests

Bioreactors were set up for the 63, 250 and 1000 mg/kg soil treatments for HD and HL in field collected soils. A second set of reactors were included which were augmented with 1000 mg/kg glucose to increase bacterial growth if the soils were nutrient depleted. The resulting carbon dioxide measurements are expressed as the CO_2 fold increase, relative to control headspace levels. Thus, larger values indicate greater soil respiration. The degree

of respiration provides an indication of the biological/microbial activity in the soil. A healthy soil ecosystem contains a dense and diverse population of microbes and invertebrates, and will have a greater volume of CO_2 respiration.

For HD-contaminated soil, respiration was unaffected at all concentrations tested, which corresponds to lack of effects noted with the soil bacterial enumeration and the results of the ECHA biomonitors. HL soils were only moderately inhibited (52% to 58%). A stronger inhibition would have been expected based on the enumeration (MPN = 0) and complete growth inhibition found with the ECHA dipsticks. It is possible that a substantial proportion of CO_2 measured resulted from abiotic soil processes, so that loss of biological activity would not be detected by measurement of total CO_2 evolution. Further work would be required to confirm this possibility.

7.0 SUMMARY OF TOXICITY TEST RESULTS

The Soil Health Index tests were designed to provide a measure of the biological health and condition of a soil and to determine a threshold for toxicity of HD and HL. The intent of the test battery was to collect a large volume of data from several trophic levels (plants, microbes and invertebrates), from which general patterns can emerge. The purpose of this study was to evaluate the sensitivity of the test battery applied to sites potentially contaminated with warfare agents, particularly, HD and HL, in order to refine the selection of tests to those of value for detection of these compounds as part of a risk assessment. The findings from this study are summarized below.

- HD did not have a major toxicological impact on any of the species tested. Moderate effects were observed with bacterial luminescence, seed emergence, and earthworm survival. The most sensitive endpoint appeared to be avoidance of HD-contaminated soil by earthworms at 160 mg/kg. This suggests that from an ecotoxicological point of view, soils contaminated with low to moderate concentrations HD would have little impact on a terrestrial ecosystem.

- HL was highly toxic to all species tested, with the exception of nematodes. Effects were observed in both direct exposure tests (seed emergence, earthworms) and extraction tests (root elongation, bacterial luminescence, algae), suggesting that contamination is biologically available to soil dwelling organisms. Toxicity is due to the lewisite component (L). From an ecological perspective, lewisite would pose a significant hazard to terrestrial communities from contamination at

low levels (less than 1 mg/kg), particularly since effects were observed for species in multiple trophic levels. The most sensitive endpoint was root elongation for lettuce with a NOEC of 0.067 mg/kg.

- Relative sensitivities of the test battery to HL were root elongation> bacterial luminescence > algae > seed emergence > earthworm survival

- HD is not efficiently recovered in aqueous extracts of contaminated soils; methanol extraction is capable of extracting HD from contaminated soils; however, biological testing at a maximum of 1 to 5% of the original methanol extract prevents bioassay detection of concentrations in soil less than 1000 mg/kg. Differences between extraction efficiency imply adsorption of HD to soil components, or extremely low aqueous solubility of the compound. Degradation/hydrolysis/volatile losses of the compound, previously thought to occur in soils, were not indicated in these tests.

- HL is recovered from soil by both aqueous and methanol extraction. The high degree of toxicity of this compound to bacterial luminescence and ease of extraction renders it a valuable tool for lewisite detection in soil. The effects of aging of soils on detection capabilities requires further investigation.

- Major differences in toxicity of HD or lewisite in the two types of soils were not noted, indicating that chemical behavior such as volatilization losses, adsorption to soil components, biological or chemical degradation are not influenced by soil constituents. These results need confirmation by testing in other soil types which offer different characteristics, to consider this statement universal for any soil type.

- HL inhibits diverse bacterial populations, as measured in the ECHA dipsticks to 26 mg/kg HL, and the loss of the natural bacterial population present in the field soil when exposed to 1000 mg/kg HL. Bacterial populations were not sensitive to HD.

8.0 COMPARISON OF RESULTS TO THE EPG ERA

The results of this investigation offer some similarities and differences to those found in the EPG ERA (Golder, 1997). Generally, Microtox and seed emergence were the most sensitive tests in the battery used for the ERA. Root elongation was somewhat less sensitive, however nematode survival was not a sensitive indicator of toxicity. The difference in sensitivity was similar for the same tests conducted using spiked samples in the laboratory.

The results for HD found here are difficult to compare to the results of the ERA. HD was not detected in the ERA, although several samples were found to have the characteristic HD odour and very high concentrations of sulphur. A toxic response was observed for samples having a very high (~46,000 mg/kg) sulphur concentration, and those having a much lower sulphur concentrations. In fact, some samples having a high sulphur concentration were associated with no significant toxicity. The only consistent potential causal factor associated with the toxicity was a low soil pH, likely resulting from the degradation of HD.

The threshold for toxicity of samples collected from areas suspected of lewisite contamination on the EPG was based on arsenic concentrations. A threshold to soil dependent organisms of about 300 mg/kg was determined. A much lower threshold of 56 µg lewisite/kg was determined using the same tests in this laboratory study. The higher threshold in the field may be due to oxidation of the arsenic in lewisite to the less toxic arsenate form and adsorption onto soil. Since a significant period of time had past from when the soil on the EPG was contaminated with lewisite to when it was assessed, the contamination had sufficient time to 'weather' resulting in a much reduced bioavailability. Thresholds for toxicity of arsenic in soil to plants and soil invertebrates published by Will and Suter (1995a,b) are 10 mg/kg and 60 mg/kg, respectively, also considerably lower than the threshold determined by the field data but much higher than the threshold determined with lewisite in this laboratory study.

The results presented here suggest that the toxicity of mustard and lewisite in the field decreases over time. The observation is particularly significant for lewisite, which was found to be a potent toxicant in these laboratory studies, but much less toxic after weathering in the field for a number of years (See Golder, 1997).

9.0 GENERAL RECOMMENDATIONS

The following list provides general recommendations for further studies based on the results of the current work presented in this report.

- Characterize the toxicity of HD and HL in soils analytically, in order to correlate toxicity to either the parent compound, or breakdown products, and to derive a

test procedure that would be sufficiently sensitive to be protective of all relevant receptors.

- Characterize the toxicity and fate of the parent compounds HD and HL breakdown products over time. This is required to determine if toxicity is due to HD, thiodigycol or other breakdown products. For HL, it is required to determine if the toxicity is due to lewisite, arsenic or specific chemical forms of arsenic. This would also provide information on predicting time to loss or reduction of toxicity, potential pathways to ecological receptors from contaminated soil and the long-term effects of HD or HL contamination.

- Investigate the potential for chemical/biological remediation of HL detected at ecologically harmful concentrations in contaminated soils.

- Confirm effect of soil constituents such as organic and clay content, bacterial population and chemical composition on fate, toxicity and behavior of HD and HL to increase confidence in prediction of effects of contaminated soils.

- Extraction efficiency of lewisite proved to be 100% under the study conditions used, however, this efficiency may be related to dose; a low concentration of lewisite may not be recovered as well as the unrealistically high concentration of HL tested for this study. The effects of dose with extraction recovery requires verification over a wide range of concentrations.

- Determine the detection limits for the ECHA dipstick for use in field screening tests for detection of lewisite-contaminated soil.

- Evaluate the degree of toxicity of HL to a variety of natural bacterial populations from a number of sources, and determine the degree of persistence of toxicity to bacterial populations or the possibility of repopulation of a contaminated site.

- Further investigation into the sensitivity of earthworm avoidance as a possible method of detection of HD

10.0 CLOSURE

We hope that this report meets your needs at this time. If you have any questions or comments, please do not hesitate to contact the undersigned.

Yours very truly,

GOLDER ASSOCIATES LTD.

Trish Miller, M.Sc.
Senior Toxicologist

Reidar Zapf-Gilje, Ph.D., P.Eng.
Principal

HydroQual Laboratories Ltd.

Darlene Linttot, M.Sc.

Stephen Goudey, Ph.D.
President

SG/TAM/kb
972-1940
J:\RPT-98\SEP\TAM-1948.doc

11.0 GENERAL REFERENCES

American Society of Testing and Materials. 1996a. Standard Guide for Conducting a Laboratory Soil Toxicity Test with Lumbricid Earthworm *Eisenia fetida*. E1676-95. ASTM vol. 11.05

American Society of Testing and Materials. 1996b. Standard Practice for Conducting Early Seedling Growth Tests. E1598-94. ASTM vol. 11.05.

Compton, J.A.F., Military Chemical And Biological Agents. Chemical And Toxicological Properties. Telford press, Caldwell N.J. 1987.

Environment Canada. 1992a. Biological Test Method: Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum*, 1992. Environ. Can. EPS1/RM/25.

Environment Canada. 1992b. Biological Test Method: Toxicity Test using Luminescent Bacteria (*Vibrio fischeri*), 1992. Environ. Can. EPS 1/RM/24.

Carter, M.R., 1993. Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton.

Greene, J.C., C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson, and W.E. Miller. 1989. Protocol for Short Term Toxicity Screening of Hazardous Waste Sites. EPA/3-88-029.

Golder, 1997. Ecological Risk Assessment and Development of Remediation Options for Selected Sites on the Experimental Proving Grounds, Defence Research Establishment Suffield. Phase I. Prepared by Golder Associates Ltd. for Defence Research Establishment Suffield, Medicine Hat, Alberta.

Kjearsgaard, A. 1973. Soils of the Suffield Military Reserve, Alberta Institute of Pedology, Report No. M-73-9.

McAndless, JM, Defence Research Establishment Suffield, personal communication, 1998.

Organization for Economic Cooperation and Development. 1993. Method 203. Terrestrial Plants Growth Test. OECD, Paris.

Stephenson, G.L., N.K. Kaushik, K.R. Solomon, T. Steele. 1997a. The predictive value of an avoidance response test with earthworms exposed to contaminated soils.

Presented at the 18th Annual Meeting of the Society for Environmental Toxicology and Chemistry, November, 1997.

Stephenson, G.L., K.R. Solomon, B. Hale. 1997b. Selection of terrestrial species for assessment of the toxicity of contaminated site soil. Presented at the 18th Annual Meeting of the Society for Environmental Toxicology and Chemistry, November, 1997.

Will, M.E. and G.W. Suter 1995a. Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Terrestrial Plants: 1995 Revisison. Prepared by the Environmental Restoration Risk Assessment Program. Prepared for the US Department of Energy, Office of Environmental Management, September, 1995.

Will, M.E. and G.W. Suter 1995b. Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Soil and Litter Invertebrates and Heterotrophic Process. Prepared by the Environmental Restoration Risk Assessment Program. Prepared for the US Department of Energy, Office of Environmental Management, September, 1995.

Table 1

Results of Toxicity Tests and Corresponding Scores for DRES Soil Sample Spiked at Nominal Mustaard Concentration of 200 mg/kg for DRES ERA

Test	Aqueous Extract	Methanol Extract	Soil	Score
Bacterial Luminescence (% control)	104	79	na	1
Algal Growth (% control)	8	15	na	na
Root Elongation (% control)				
lettuce	96	40	na	na
northern wheatgrass	83	0	na	1
Nematode Survival (0-poor; 2-good)	2	2	na	1
Seed Emergence (% control)				
lettuce	na	na	95	na
northern wheatgrass	na	na	45	3
Soil Respiration	na	na	40	3
Bacterial Counts	na	na	6	3

na - not applicable

Table 2
Chemical Parameters on Extracts

Soil Type	pH	Conductivity (µS/cm)
Artificial Soil Control	4.0	80
Field Soil Control	7.4	608
Mustard HD in Artificial Soil	3.2	589
Mustard HL (lewisite) in Artificial Soi	3.6	511
Mustard HD in Field Soil	6.1	822
Mustard HL (lewisite) in Field Soil	7.4	861

Table 3
Bacterial Luminescence Test Results of Pure Materials

Mustard (HD) in mg/L

	tested < 0.5 h post-preparation not pH adjusted		tested 1 h post-preparation pH adjusted to 6.6		tested 24 h post-preparation not pH adjusted		tested 8 days post-preparation not pH adjusted	
	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits
IC20	76	36-160	14	7-27	14	13-17	6	4-8
IC50	100	55-190	58	40-84	21	14-32	19	16-22

Mustard Lewisite (HL) in mg/L

	tested 5 h post-preparation not pH adjusted		tested 5 h post-preparation pH adjusted to 7.6		tested 24 h post-preparation not pH adjusted		tested 8 days post-preparation not pH adjusted	
	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits	ICx mg/L	95% confidence limits
IC20	0.010	0.009-0.011	0.010	0.008-0.013	0.014	0.011-0.017	0.014	0.012-0.017
IC50	0.027	0.026-0.029	0.032	0.029-0.036	0.035	0.032-0.038	0.038	0.034-0.043

Comments pH of 1000 mg/L mustard stock solution = 2.2
pH of 1000 mg/L mustard HL stock solution = 1.8

Table 4
Bacterial Luminescence Test Results of Fortified Soils, Aqueous and Methanol Extracts

Mustard (HD) in Artificial Soil

	Aqueous Extract		Methanol Extract	
	ICx %	95% confidence limits	ICx %	95% confidence limits
IC20	31	13-77	1.4	0.81-2.3
IC50	41	21-79	4.9	1.8-14

Mustard (HD) in Field Soil

	Aqueous Extract		Methanol Extract	
	ICx %	95% confidence limits	ICx %	95% confidence limits
IC20	53	39-72	2.4	1.5-3.7
IC50	> 91	n/a	5.6	2.3-14

Mustard Lewisite (HL) in Artificial Soil

	Aqueous Extract		Methanol Extract	
	ICx %	95% confidence limits	ICx %	95% confidence limits
IC20	0.0050	0.0047-0.0054	0.0039	0.0034-0.0044
IC50	0.014	0.014-0.015	0.011	0.010-0.012

Mustard Lewisite (HL) in Field Soil

	Aqueous Extract		Methanol Extract	
	ICx %	95% confidence limits	ICx %	95% confidence limits
IC20	0.0051	0.0047-0.0055	0.0075	0.0052-0.011
IC50	0.016	0.016-0.017	0.019	0.015-0.025

Comments: all soil extracts were tested without pH adjustment
results are presented as percent dilution of the extract
artificial soil deionized water and methanol extraction controls; Microtox results = IC20 > 91%, IC50 > 91%
field soil deionized water and methanol extraction controls; Microtox results = IC20 > 91%, IC50 > 91%

Table 5
Results of Bacterial Browth Test (ECHA Biomonitors) Exposed to Fortified Soils

Mustard HD in Artificial Soil		Mustard HD in Field Soil	
Treatment (mg/Kg)	**Result**	**Treatment (mg/Kg)**	**Result**
Control	1	Control	2
26	2	26	2
64	2	64	2
160	2	160	2
400	2	400	2
1000	2	1000	2

Mustard HL (lewisite) in Artificial Soil		Mustard HL (lewisite) in Field Soil	
Treatment (mg/Kg)	**Result**	**Treatment (mg/Kg)**	**Result**
Control	1	Control	2
26	0	26	0
64	0	64	0
160	0	160	0
400	0	400	0
1000	0	1000	0

Comments: 0, no growth; 1, moderate growth; 2 abundant growth

Table 6
Results of Seedling Emergence Tests

LETTUCE — **Mustard in Artificial Soil**

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	107	102	107	**105**	3	
hctl	96	96	107	**100**	6	
26	107	102	96	**102**	5	
64	102	102	91	**98**	6	
160	102	86	91	**93**	8	
400	96	102	102	**100**	3	
1000	91	91	43	**75**	28	no roots, reduced shoot height

Test Endpoints:		confid. limits.		
LC25:	819	400-1000	**NOEC:**	1000
LC50:	> 1000		**LOEC:**	> 1000

ALFALFA — **Mustard in Artificial Soil**

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	79	136	107	**107**	29	
hctl	107	114	79	**100**	19	
26	114	107	71	**98**	23	
64	100	100	114	**105**	8	
160	121	79	79	**93**	25	
400	107	93	93	**98**	8	
1000	29	64	71	**55**	23	no roots, reduced shoot height

Test Endpoints:		confid. limits.		
LC25:	668	458-913	**NOEC:**	4000
LC50:	> 1000		**LOEC:**	1000

Table 6
Results of Seedling Emergence Tests

NORTHERN WHEATGRASS

Mustard in Artificial Soil

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	88	124	80	**98**	24	
hctl	88	102	110	**100**	11	
26	73	59	102	**78**	22	
64	117	102	102	**107**	8	
160	102	73	80	**85**	15	
400	80	102	88	**90**	11	
1000	73	22	59	**51**	26	no roots, reduced shoot height

Test Endpoints:		confid. limits.		
LC25:	618	400-1000	NOEC:	400
LC50:	> 1000		LOEC:	1000

LETTUCE

Mustard in Field Soil

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	103	145	134	**128**	22	
hctl	103	62	134	**100**	36	
26	31	83	62	**59**	26	
64	83	134	124	**114**	27	
160	145	166	145	**152**	12	
400	166	197	186	**183**	16	
1000	197	134	83	**138**	57	no roots, stunted shoots, just emerged

Test Endpoints:		confid. limits.		
LC25:	> 1000		NOEC:	1000
LC50:	> 1000		LOEC:	> 1000

Table 6
Results of Seedling Emergence Tests

ALFALFA

Mustard In Field Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	130	100	100	**110**	17	
hctl	120	80	100	**100**	20	
26	110	80	140	**110**	30	
64	130	140	100	**123**	21	
160	70	130	150	**117**	42	
400	140	90	120	**117**	25	
1000	140	120	120	**127**	12	no roots, stunted shoots, just emerged

Test Endpoints:		confid. limits.		
LC25:	> 1000	NOEC:	1000	
LC50:	> 1000	LOEC:	> 1000	

NORTHERN WHEATGRASS

Mustard in Field Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	100	100	144	**115**	26	
hctl	133	67	100	**100**	33	
26	100	133	78	**104**	28	
64	67	133	78	**93**	36	
160	111	144	122	**126**	17	
400	89	122	33	**81**	45	
1000	0	0	0	**0**	0	seeds not germinated, no shoots

Test Endpoints:		confid. limits.		
LC25:	387	178-550	NOEC:	400
LC50:	572	354-700	LOEC:	1000

Table 6
Results of Seedling Emergence Tests

LETTUCE — **Mustard HL (lewisite) in Artificial Soil**

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	107	102	107	105	3	
hctl	96	96	107	100	6	
26	16	86	70	57	36	no roots, reduced shoot height
64	38	32	27	32	5	no roots, stunted shoots, just emerged
160	0	0	0	0	0	
400	0	0	0	0	0	
1000	0	0	0	0	0	

Test Endpoints:		confid. limits.		
LC25:	17	8-34	NOEC:	< 26
LC50:	36	17-52	LOEC:	26

ALFALFA — **Mustard HL (lewisite) in Artificial Soil**

Treatment mg/Kg	% Emergence			Average	SD	comment
ctl	79	136	107	107	29	
hctl	107	114	79	100	19	
26	71	114	100	95	22	
64	7	7	7	7	0	no roots, stunted shoots, just emerged
160	7	7	7	7	0	no roots, stunted shoots, just emerged
400	0	0	0	0	0	
1000	0	0	0	0	0	

Test Endpoints:		confid. limits.		
LC25:	33	26-36	NOEC:	26
LC50:	45	39-47	LOEC:	64

Table 6
Results of Seedling Emergence Tests

NORTHERN WHEATGRASS

Mustard HL (lewisite) in Artificial Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	88	124	80	98	24	
hctl	88	102	110	100	11	
26	73	51	73	66	13	
64	51	37	59	49	11	no roots, stunted shoots, just emerged
160	0	7	0	2	4	no roots, stunted shoots, just emerged
400	0	0	0	0	0	
1000	0	0	0	0	0	

Test Endpoints:		confid. limits.			
	LC25:	19	15-28	NOEC:	< 26
	LC50:	60	39-83	LOEC:	26

Table 6. cont'd

LETTUCE

Mustard HL (lewisite) in Field Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	103	145	134	128	22	
hctl	134	103	62	100	36	
26	166	176	186	176	10	
64	31	10	10	17	12	no roots, stunted shoots, just emerged
160	0	0	0	0	0	
400	0	0	0	0	0	
1000	0	0	0	0	0	

Test Endpoints:					
	LC25:	37	36-38	NOEC:	26
	LC50:	48	46-50	LOEC:	64

Table 6
Results of Seedling Emergence Tests

ALFALFA — Mustard HL (lewisite) in Field Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	109	73	91	**91**	18	
hctl	118	91	91	**100**	16	
26	155	136	82	**124**	38	
64	109	109	118	**112**	5	
160	27	9	18	**18**	9	no roots, stunted shoots, just emerged
400	0	0	9	**3**	5	
1000	0	0	0	**0**	0	

Test Endpoints:		confid. limits.			
	LC25:	89	80-95	NOEC:	64
	LC50:	120	112-127	LOEC:	160

NORTHERN WHEATGRASS — Mustard HL (lewisite) in Field Soil

Treatment mg/Kg		% Emergence		Average	SD	comment
ctl	100	100	144	**115**	26	
hctl	133	67	100	**100**	33	
26	156	133	89	**126**	34	
64	167	89	122	**126**	39	
160	33	56	67	**52**	17	no roots, stunted shoots, just emerged
400	0	0	0	**0**	0	
1000	0	0	0	**0**	0	

Test Endpoints:		confid. limits.			
	LC25:	104	64-123	NOEC:	160
	LC50:	149	123-183	LOEC:	400

Table 7
Results of Root Elongation Tests with Extracts of Fotified Soils

LETTUCE
Mustard in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	95	34
hctl	100	19
2.6	90	16
6.4	83	17
16	100	24
40	116	21
100	90	18

Test Endpoints:	95% confid.		
LC25:	> 100	NOEC:	100
LC50:	> 100	LOEC:	> 100

LETTUCE
Mustard in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	93	30
hctl	100	14
0.026	100	14
0.064	100	15
0.16	84	28
0.40	101	17
1.0	96	21

Test Endpoints:	95% confid.		
LC25:	> 1.0	NOEC:	1.0
LC50:	> 1.0	LOEC:	> 1.0

ALFALFA
Mustard in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	95	36
hctl	100	22
2.6	76	52
6.4	88	54
16	49	47
40	72	32
100	70	45

Test Endpoints:	95% confid.		
LC25:	> 100	NOEC:	100
LC50:	> 100	LOEC:	> 100

ALFALFA
Mustard in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	78	27
hctl	100	28
0.026	72	31
0.064	65	47
0.16	82	28
0.40	73	32
1.0	77	42

Test Endpoints:	95% confid.		
LC25:	> 1.0	NOEC:	1.0
LC50:	> 1.0	LOEC:	> 1.0

Table 7
Results of Root Elongation Tests with Extracts of Fotified Soils

NORTHERN WHEATGRASS
Mustard in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	114	43
hctl	100	22
2.6	90	38
6.4	109	17
16	115	19
40	82	46
100	39	52

Test Endpoints:		95% confid.		
LC25:	47	29-64	NOEC:	40
LC50:	82	40-100	LOEC:	100

NORTHERN WHEATGRASS
Mustard in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	97	44
hctl	100	23
0.026	100	23
0.064	121	15
0.16	97	32
0.40	110	57
1.0	116	33

Test Endpoints:		95% confid.		
LC25:	> 1.0		NOEC:	1.0
LC50:	> 1.0		LOEC:	> 1.0

LETTUCE
Mustard in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	68	21
hctl	100	17
2.6	72	19
6.4	69	32
16	83	15
40	87	10
100	88	17

Test Endpoints:		95% confid.		
LC25:	> 100		NOEC:	100
LC50:	> 100		LOEC:	> 100

LETTUCE
Mustard in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	87	16
hctl	100	32
0.026	81	31
0.064	78	44
0.16	74	18
0.40	80	21
1.0	76	21

Test Endpoints:		95% confid.		
LC25:	> 1.0		NOEC:	1.0
LC50:	> 1.0		LOEC:	> 1.0

Table 7

Results of Root Elongation Tests with Extracts of Fotified Soils

ALFALFA

Mustard in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	114	44
hctl	100	53
2.6	89	53
6.4	105	45
16	109	44
40	106	40
100	94	17

Test Endpoints:		95% confid.		
LC25:	> 100		NOEC:	100
LC50:	> 100		LOEC:	> 100

ALFALFA

Mustard in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	68	48
hctl	100	50
0.026	143	10
0.064	129	12
0.16	80	81
0.40	101	48
1.0	48	43

Test Endpoints:		95% confid.		
LC25:	0.49	0.15-0.67	NOEC:	0.4
LC50:	0.86	0.4-1.0	LOEC:	1.0

NORTHERN WHEATGRASS

Mustard in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	119	18
hctl	100	28
2.6	144	21
6.4	119	49
16	112	24
40	118	31
100	89	34

Test Endpoints:		95% confid.		
LC25:	> 100		NOEC:	100
LC50:	> 100		LOEC:	> 100

NORTHERN WHEATGRASS

Mustard in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	81	51
hctl	100	35
0.026	86	27
0.064	101	15
0.16	93	42
0.40	35	69
1.0	99	15

Test Endpoints:		95% confid.		
LC25:	> 1.0		NOEC:	1.0
LC50:	> 1.0		LOEC:	> 1.0

Table 7
Results of Root Elongation Tests with Extracts of Fotified Soils

LETTUCE

Mustard HL in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	109	27
hctl	100	32
0.004	100	34
0.011	104	25
0.027	96	26
0.067	69	52
0.17	37	36
0.42	24	27
1.04	9	63
2.6	2	0

Test Endpoints:		95% confid.		
LC25:	0.056	0.026-0.10	NOEC:	0.026
LC50:	0.12	0.056-0.15	LOEC:	0.16

LETTUCE

Mustard HL in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	101	15
hctl	100	29
0.026	95	20
0.064	67	40
0.16	33	30
0.40	11	46
1.0	4	181

Test Endpoints:		95% confid.		
LC25:	0.053	0.034-0.082	NOEC:	0.16
LC50:	0.11	0.068-0.13	LOEC:	0.40

ALFALFA

Mustard HL in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	118	61
hctl	100	35
0.004	103	37
0.011	109	46
0.027	104	36
0.067	116	38
0.17	87	36
0.42	100	44
1.04	83	91
2.6	26	30

Test Endpoints:		95% confid.		
LC25:	1.1	0.56-1.5	NOEC:	1
LC50:	1.8	0.76-2.1	LOEC:	2.6

ALFALFA

Mustard HL in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	104	39
hctl	100	14
0.026	56	56
0.064	64	48
0.16	66	55
0.40	48	46
1.0	27	57

Test Endpoints:		95% confid.		
LC25:	0.017	0.012-0.17	NOEC:	0.026
LC50:	0.37	0.095-0.68	LOEC:	0.16

Table 7
Results of Root Elongation Tests
with Extracts of Fotified Soils

NORTHERN WHEATGRASS
Mustard HL in Artificial Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	113	35
hctl	100	34
0.004	128	23
0.011	129	21
0.027	132	33
0.067	130	24
0.17	78	22
0.42	108	31
1.04	72	55
2.6	46	54

Test Endpoints:		95% confid.		
LC25:	0.48	0.14-0.99	NOEC:	1.0
LC50:	1.6	1.0-2.6	LOEC:	2.6

NORTHERN WHEATGRASS
Mustard HL in Artificial Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	84	47
hctl	100	47
0.026	131	51
0.064	140	30
0.16	113	44
0.40	92	55
1.0	54	66

Test Endpoints:		95% confid.		
LC25:	0.37	0.15-0.68	NOEC:	0.400
LC50:	0.85	0.40-1.0	LOEC:	1.00

Table 7
Results of Root Elongation Tests with Extracts of Fotified Soils

LETTUCE
Mustard HL in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	91	20
hctl	100	21
0.004	91	33
0.011	78	36
0.027	66	48
0.067	78	27
0.17	59	34
0.42	32	33
1.04	17	33
2.6	7	94

Test Endpoints:		95% confid.		
LC25:	0.06	0.009-0.14	NOEC:	0.067
LC50:	0.24	0.16-0.31	LOEC:	0.16

LETTUCE
Mustard HL in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	119	17
hctl	100	20
0.0102	98	20
0.026	82	55
0.064	47	46
0.16	38	24
0.40	21	48
1.0	6	101

Test Endpoints:		95% confid.		
LC25:	0.036	0.016-0.046	NOEC:	0.026
LC50:	0.063	0.029-0.11	LOEC:	0.064

ALFALFA
Mustard HL in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	154	23
hctl	100	33
2.560	111	24
6.40	72	35
16.00	33	0
40	0	0
100	0	0

Test Endpoints:		95% confid.		
LC25:	5.6	3.0-7.9	NOEC:	6.4
LC50:	10	6.9-12.8	LOEC:	16

ALFALFA
Mustard HL in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	66	24
hctl	100	18
0.026	122	9
0.064	108	12
0.16	77	66
0.40	85	48
1.0	40	43

Test Endpoints:		95% confid.		
LC25:	0.15	0.11-0.52	NOEC:	0.40
LC50:	0.78	0.4-1.0	LOEC:	1.0

Table 7
Results of Root Elongation Tests with Extracts of Fotified Soils

NORTHERN WHEATGRASS
Mustard HL in Field Soil - Aqueous Extract

Treatment % extract	% Control	% cv
ctl	150	37
hctl	100	49
2.560	64	41
6.40	37	44
16.00	43	0
40	43	0
100	0	0

Test Endpoints:		95% confid.		
LC25:	1.8	1.2-4.7	NOEC:	1.0
LC50:	4.7	5.6-6.4	LOEC:	2.6

NORTHERN WHEATGRASS
Mustard LHL in Field Soil - Methanol Extract

Treatment % extract	% Control	% cv
ctl	101	35
hctl	100	30
0.010	84	22
0.026	69	43
0.064	95	23
0.16	76	39
0.40	50	74
1.0	65	46

Test Endpoints:		95% confid.		
LC25:	0.17	0.064-1.0	NOEC:	0.160
LC50:	> 1.0		LOEC:	0.40

Table 8

Results of Algal Growth Inihibition Tests with Extracts of Fortified Soils

Mustard in Artificial Soil

Deionized Water Extract

Treatment (%)	% Control	CV(%)
ctl	100	30
0.41	108	55
1.0	133	11
2.6	157	8
6.4	164	11
16	157	4
40	0	0
100	0	0
Test Endpoints:		
IC25	22	16-40
IC50	28	16-40
NOEC:	16	
LOEC:	40	

Methanol Extract

Treatment (%)	% Control	CV(%)
ctl	100	18
0.0041	99	13
0.010	85	8
0.03	82	25
0.06	109	17
0.16	84	12
0.40	84	21
1.0	65	24
Test Endpoints:		
IC25:	0.68	0.4-1.0
IC50:	> 1	
NOEC:	0.4	
LOEC:	1.0	

Mustard in Field Soil

Deionized Water Extract

Treatment (%	% Control	CV(%)
ctl	100	13
0.41	95	19
1.0	111	22
2.6	126	12
6.4	135	5
16	117	12
40	92	9
100	62	21
Test Endpoints:		
IC25	53	37-67
IC50	> 100	16-40
NOEC:	40	
LOEC:	100	

Methanol Extract

Treatment (%)	% Control	CV(%)
ctl	100	17
0.0041	61	17
0.010	58	22
0.03	87	9
0.06	65	24
0.16	46	30
0.40	76	33
1.0	75	33
Test Endpoints:		
IC25:	0.088	0.082-0.016
IC50:	> 1.0	
NOEC:	1.0	
LOEC:	> 1.0	

Mustard Lewisite (HL) in Artificial Soil

Deionized Water Extract

Treatment (%)	% Control	CV(%)
ctl	100	17
0.011	136	22
0.027	154	11
0.07	136	14
0.17	20	84
0.42	0	0
1.0	0	0
2.6	0	0
Test Endpoints:		
IC25	0.097	0.089-0.10
IC50	0.13	0.12-0.14
NOEC:	0.067	
LOEC:	0.17	

Methanol Extract

Treatment (%)	% Control	CV(%)
ctl	100	18
0.0041	99	13
0.010	85	8
0.03	82	25
0.06	109	17
0.16	84	12
0.40	84	21
1.0	65	24
Test Endpoints:		
IC25:	0.071	0.020-0.11
IC50:	0.1	
NOEC:	0.064	
LOEC:	0.2	

Mustard Lewisite (HL) in Field Soil

Deionized Water Extract

Treatment (%	% Control	CV(%)
ctl	100	19
0.011	114	19
0.027	141	22
0.07	138	12
0.17	107	5
0.42	0	12
1.0	0	9
2.6	0	21
Test Endpoints:		
IC25	0.23	0.21-0.23
IC50	0.30	0.27-0.30
NOEC:	0.17	
LOEC:	0.42	

Methanol Extract

Treatment (%)	% Control	CV(%)
ctl	100	14
0.0041	68	8
0.010	73	24
0.026	74	31
0.064	51	41
0.16	0	0
0.40	0	0
1.0	0	0
Test Endpoints:		
IC25:	0.055	0.045-0.069
IC50:	0.077	0.067-0.082
NOEC:	0.026	
LOEC:	0.064	

GOLDER ASSOCIATES LTD.

HYDROQUAL LABORATORIES LTD.

Table 9

Results of 7-Day Earthworm Survival Tests with Fortified Soils

Mustard in Artificial Soil

Treatment mg/Kg	% Survival			Average	SD	comment
ctl	100	100	100	100	0	
hctl	100	100	90	97	6	
26	90	100	100	97	6	
64	100	100	100	100	0	
160	100	100	100	100	0	avoidance, lethargic
400	100	100	100	100	0	avoidance, lethargic
1000	100	100	100	100	0	avoidance, lethargic

Test Endpoints:		95% conf.		
LC25:	> 100	NOEC:	100	
LC50:	> 100	LOEC:	> 100	

Mustard in Field Soil

Treatment mg/Kg	% Survival			Average	SD	comment
ctl	100	100	100	100	0	
hctl	100	100	90	97	6	
26	100	100	100	100	0	
64	100	100	100	100	0	
160	100	100	100	100	0	avoidance
400	100	100	100	100	0	avoidance, stressed
1000	100	100	100	100	0	avoidance, stressed

Test Endpoints:		95% conf.		
LC25:	> 100	NOEC:	100	
LC50:	> 100	LOEC:	> 100	

Mustard HL (lewisite) in Artificial Soil

Treatment mg/Kg	% Survival			Average	SD	comment
ctl	100	100	100	100	0	
hctl	100	100	100	100	0	
26	100	100	100	100	0	complete avoidance
64	100	100	100	100	0	complete avoidance
160	0	0	0	0	0	
400	0	0	0	0	0	
1000	0	0	0	0	0	

Test Endpoints:			95% conf.		
LC25:	88	64-160	NOEC:	64	
LC50:	112	64-160	LOEC:	160	

Mustard HL (lewisite) in Field Soil

Treatment mg/Kg	% Survival			Average	SD	comment
ctl	100	100	100	100	0	
hctl	100	100	100	100	0	
26	100	100	100	100	0	
64	100	100	100	100	0	
160	90	0	0	30	52	avoidance
400	0	80	0	27	46	avoidance, stressed
1000	0	0	0	0	0	

Test Endpoints:			95% conf.		
LC25:	98	88-124	NOEC:	64	
LC50:	133	112-454	LOEC:	160	

note: 95% conf. = 95% confidence limits; SD = standard deviation

Table 10

Results of Nematode Survival Tests in Extracts of Fortified Soils

Mustard in Artificial Soil

Deionized Water Extract

Treament %	% Control
ctl	100
hctl	100
1.02	104
2.56	43
6.4	104
16	93
40	98
100	88
Test Endpoints:	
LC25:	> 100
LC50:	> 100
NOEC:	100
LOEC:	> 100

Methanol Extract

Treament	% Control
ctl	112
hctl	100
0.026	32
0.064	95
0.16	126
0.4	110
1	95
Test Endpoints:	
LC25:	> 100
LC50:	> 100
NOEC:	100
LOEC:	> 100

Mustard in Field Soil

Deionized Water Extract

Treatment	% Control
ctl	33
hctl	100
1.024	107
2.56	104
6.4	109
16	110
40	36
100	103
Test Endpoints:	
LC25:	>100
LC50:	> 100
NOEC:	100
LOEC:	> 100

Methanol Extract

Treatment	% Control
ctl	30
hctl	100
0.026	24
0.064	0
0.16	60
0.4	80
1	60
Test Endpoints:	
LC25:	> 100
LC50:	> 100
NOEC:	100
LOEC:	> 100

Mustard HL (lewisite) In Artificial Soil

Deionized Water Extract

Treatment	% Control	
ctl	89	
hctl	100	
1.02	85	
2.56	84	
6.4	87	
16	59	
40	43	
100	3	
Test Endpoints:		95% c.l.
LC25:	11	5-40
LC50:	29	13-57
NOEC:	16	
LOEC:	40	

Methanol Extract

Treatment	% Ctl
ctl	64
hctl	100
0.010	8
0.026	33
0.064	46
0.16	100
0.4	106
1	63
Test Endpoints:	
LC25:	> 1.0
LC50:	> 1.0
NOEC:	1
LOEC:	> 1.0

GOLDER ASSOCIATES LTD.

Mustard HL (lewicite) in Field Soil

Deionized Water Extract

Treatment	% Control	
ctl	91	
hctl	100	
1.024	100	
2.56	71	
6.4	63	
16	77	
40	40	
100	7	
Test Endpoints:		95% c.l.
LC25:	5	3-19
LC50:	37	19-60
NOEC:	16	
LOEC:	40	

Methanol Extract

Treatment	% Control
ctl	75
hctl	75
0.026	75
0.064	50
0.16	0
0.4	50
1	95
Test Endpoints:	
LC25:	> 1.0
LC50:	> 1.0
NOEC:	1
LOEC:	> 1.0

HYDROQUAL LABORATORIES LTD.

Table 11
Carbon Dioxide Measurements from Bioreactors of Fortified Soils

Mustard in Field Soil (Unamended)

Treatment	Replicate			Average	SD	% Ctl
	A	B	C			
hctl	10748	2254	3089	**5364**	4682	100
63	13469	5977		**9723**	5298	181
250	16393	10715	7463	**11524**	4520	215
1000	6257	5410	10820	**7496**	2910	140

Mustard HL (lewisite) in Field Soil (Unamended)

Treatment	Replicate			Average	SD	% Ctl
	A	B	C			
hctl	10748	2254	3089	**5364**	4682	100
63	1739	2950	1197	**1962**	898	37
250	7288	2934	1701	**3974**	2935	74
1000	2197	2302	2292	**2264**	58	42

Mustard in Field Soil (Amended)

Treatment	Replicate			Average	SD	% Ctl
	A	B	C			
hctl	2597	5052	5760	**4470**	1660	100
63	1692	5892	2028	**3204**	2334	72
250	2716	7466	5993	**5392**	2431	121
1000	6974	5547	10086	**7536**	2321	169

Mustard HL (lewisite) in Field Soil (Amended)

Treatment	Replicate			Average	SD	% Ctl
	A	B	C			
hctl	2597	5052	5760	**4470**	1660	100
63	3195	5424	1964	**3528**	1754	79
250	3440	1275	1703	**2139**	1147	48
1000	2342	1757	2519	**2206**	399	49

Note: Amended soils refer to an addition of glucose to soils at a rate of 1000 mg/Kg.

METHOD

MICROBES

- bacterial luminescence (water and methanol extracts)
- microbial characterization - total heterotrophic bacteria
- bacterial growth - ECHA Biomonitors (soil)

PLANTS

- 5 day root elongation – lettuce, alfalfa and northern wheatgrass (water and methanol extracts)
- seedling emergence – lettuce, alfalfa and northern wheatgrass (soil)
- algal growth inhibition (water and methanol extracts)

INVERTEBRATE

- Nematode survival
- Earthworm survival

COMMUNITY PROCESSES

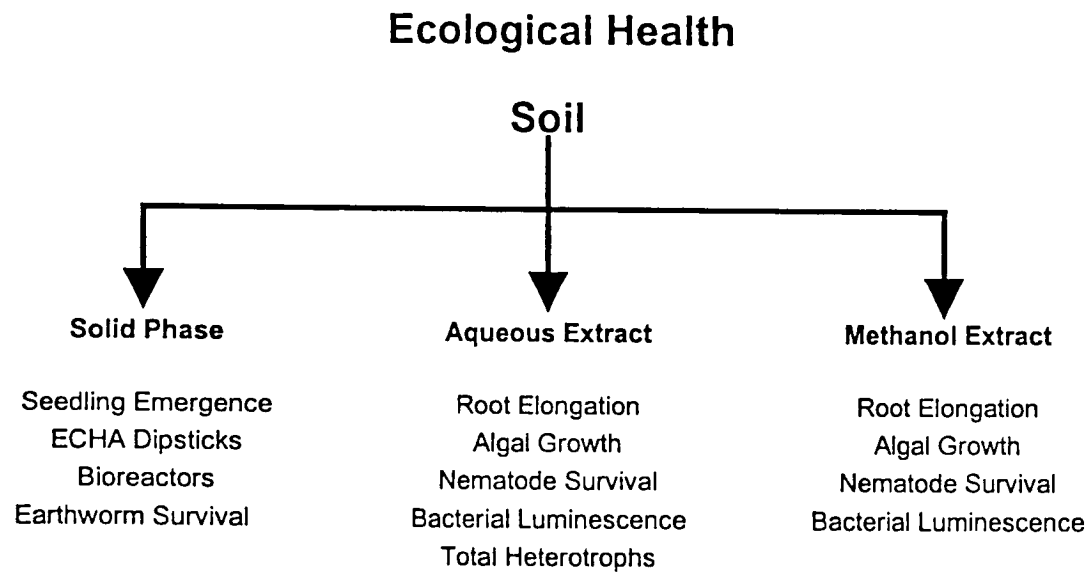
- soil respiration (soil)

HydroQual Laboratories Ltd.

Golder Associates

Test Methods Incorporated into the Study Design

Figure 1



HydroQual Laboratories Ltd.

Golder Associates

Flow Chart for Processing Test Soils

Figure **2**

APPENDIX I

CHEMICAL COMPOSITION OF ARTIFICIAL SOIL

Artificial Soil Chemical Composition

MATERIAL	RATIO
2.36 mm screened sphagnum peat	1
colloidal kaolonite clay	2
grade 70 silica sand, Winroc	7

Note: the artificial soil is composed of 1 part peat, 2 parts clay, and 7 parts sand by weight

DOCUMENT CONTROL DATA

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.)

Golder Associates Ltd
500-4260 Still Creek Drive
Burnaby, BC V5C 6C6

2. SECURITY CLASSIFICATION (overall security classification of the document, including special warning terms if applicable)

Unclassified

3. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title).

The Toxicity of Mustard and Mustard Lewisite to Terrestrial Organisms (U)

4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

Miller, Trish; Goudey, Stephen and Zapf-Gilje, Reidar

5. DATE OF PUBLICATION (month and year of publication of document)

September 1998

6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 64

6b. NO. OF REFS (total cited in document) 15

7. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Contract Report

8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)

Defence Research Establishment Suffield, PO Box 4000, Station Main, Medicine Hat, AB, T1A 8K6

9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)

9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)

W7702-5-0382

10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)

DRDC Suffield CR 2005-198

10b. OTHER DOCUMENT NOs. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

11. DOCUMENT AVAILABILITY (any limitations on further dissemination of the document, other than those imposed by security classification)

- (x) Unlimited distribution
- ( ) Distribution limited to defence departments and defence contractors; further distribution only as approved
- ( ) Distribution limited to defence departments and Canadian defence contractors; further distribution only as approved
- ( ) Distribution limited to government departments and agencies; further distribution only as approved
- ( ) Distribution limited to defence departments; further distribution only as approved
- ( ) Other (please specify):

12. DOCUMENT ANNOUNCEMENT (any limitation to the bibliographic announcement of this document. This will normally corresponded to the Document Availability (11). However, where further distribution (beyond the audience specified in 11) is possible, a wider announcement audience may be selected).

Unlimited

13. ABSTRACT (a brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C) or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual).

Chemical warfare agents, specifically mustard (HD) and mustard-lewisite (HL) mixtures have been used and stored in defence research and training establishments in Canada and abroad. HydroQual Laboratories Ltd. was contracted through Golder Associates Ltd. (Burnaby) to evaluate the toxicity of HD and HL to soil-dwelling organisms for Defence Research Establishment Suffield (DRES). The toxicity of HD and HL to terrestrial organisms was evaluated by applying soil health index tests (SHI) to two types of soils fortified with known quantities of HD and HL. Several concentrations were used to establish a dose-response relationship. The tests included root elongation and seedling emergence (lettuce, alfalfa and northern wheatgrass), soil respiration, bacterial growth (ECHA biomonitors), total heterotrophic bacteria, nematode survival, earthworm survival, algal growth inhibition, and bacterial luminescence. Tests were done on both water and methanol extracts of the soils. These solvents also permitted resolution of the presence and availability of contaminants with different physical and chemical properties.

The soil samples spiked with HD did not have a strong toxicological impact on the microbial, plant or invertebrate species tested. The most sensitive endpoint noted was earthworm avoidance with a no effect concentration of 160 mg/kg. Mustard-lewisite applied to soils was highly toxic to all trophic levels tested, for both direct soil exposure tests, and aqueous and methanol extracts. The most sensitive endpoint was root elongation for the lettuce with a no effect concentration of 0.067 mg/kg. The results indicated that the soil health index test battery would provide a valuable tool for detection of agent-contaminated soils, and suggest that low levels of soil freshly contaminated with HL would pose a significant risk to soil-dependent receptors.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifies, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

Mustard
Lewisite
HD
L
Mustard-Lewisite
Toxicities
Terrestrial Organisms
Soils
Microbial
Plants
Invertebrate
Bacterial Luminescence
Echa Biomonitor
Seedling Emergence
Root Elongation
Algal Growth
Earthworm
Nematode
Soil Tests

Defence R&D Canada

Canada's Leader in Defence
and National Security
Science and Technology

R & D pour la défense Canada

Chef de file au Canada en matière
de science et de technologie pour
la défense et la sécurité nationale

DEFENCE R&D DÉFENSE

www.drdc-rddc.gc.ca